LA-UR-21-21739
Approved for public release; distribution is unlimited.

Title: Diagnostic Testing for COVID-19 Bridging Study for QIAamp Viral RNA Extraction vs Beckman RNAdvance vs Thermofisher MagMAX

Author(s): Hollander, Attelia Dawn

Intended for: Report

Issued: 2021-02-23

Los Alamos National Laboratory
Biological Agent Testing Laboratory

Diagnostic Testing for COVID-19
Bridging Study for QIAamp Viral RNA Extraction vs Beckman RNAdvance vs Thermofisher MagMAX

February 15, 2021

TABLE OF CONTENTS

Executive Summary

This report describes testing that was performed by LANL's Biological Agent Testing Lab (BATL) to validate modifications to the CDC EUA 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA-CDC-nCoV-IFU). BATL intends to implement the modifications to increase thoughput for daily testing . BATL validated the viral RNA extraction process, using the orignial component, QIAamp Viral RNA Mini Kit (Cat # 52906) and the new components, Beckman Coulter magnetic 96-well plate RNAdvance Viral kit (Cat # C63510), Thermofisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat # A48310). Equivalency was demonstrated between the original component and the Beckman Coulter magnetic 96-well plate RNAdvance Viral kit (Cat # C63510). Equivalency was also demonstrated between the original component and the Thermofisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat # A48310). Subsequently, substitution of the original component with either of these kits for viral RNA extraction increased BATL's extraction capability from 100 samples per day to 279 samples per day.

1.0 Introduction and Purpose

LANL's Biological Agent Testing Laboratory (BATL) performs testing for SARS-CoV-2 for LANL employees, and provides surge capacity testing for the State of New Mexico. To increase the testing capabilities of BATL, we conducted a bridging study to validate proposed changes to the CDC-EUA and establish equivalency between the original component for viral RNA extraction and two alternative new components listed on the FDA website as being reasonable alternatives (website). BATL's original viral RNA extraction process used the CDC EUA assay's QIAamp viral RNA mini kit, which is low throughput. BATL needed to increase RNA extraction efficiency by moving to a 96-well RNA extraction format. This report summarizes the methods BATL used to validate the higher throughput alternatives and the results demonstrating equivalency.

2.0 Evaluation Process

2.1 Range Finding

BATL selected a known positive clinical sample collected and provided by the New Mexico Department of Health Scientific Laboratory Division (NM DoH SLD) as a sample spike, that fell within a Ct Range of 20-25. A set of known negative samples were pooled together to provide the sample matrix. The detection range of the original CDC-EUAassay was determined using a dilution series produced by spiking the positive sample into the pooled negative sample matrix. Serial dilution ranges were prepared depending on the initial Ct, using the pooled negative clinical samples as a diluent. A dilution series of five concentrations were run in triplicate beginning with an approximate expected Ct of 30.

For example:

If Initial Ct = 20.
1:10 _expected Ct 23.3
1:100_ expected Ct 26.6
1:1000_ expected Ct 30
1:10000_ expected Ct 33.3
1:100000_ expected Ct 36.6
1:1000000_ expected Ct 40
1:10000000_ expected Ct 43.3

Once the range was determined using the original extraction method and assays, we then performed the Limit of detection (LOD) study described next, to determine equivalency between the extraction kits.

2.2 Limit of Detection (LOD)

The purpose of this experiment was to identify the lowest concentration for which a set of triplicate reactions generate positive results, using a dilution series of the virus in clinical matrix. This would be the target dilution for LOD. The dilution window was further tested with the new extraction method and assays. Three concentrations consisting of the target dilution identified during the range finding as well as one 3-fold dilution above and below the identified target dilution, were tested. Three extraction methods were compared in this study. QIAamp Viral RNA mini kit, Beckman Coulter RNAdvance, and the Thermofisher MagMAX. For each extraction method each dilution was extracted 10 times. Each extraction was be run in duplicate, using the IDT Multiplex SARS-Cov2 detection assays, thus yielding a total of 20 tests for a single dilution. The two extraction kits were considered to have equivalent performance if the resultant Limit of Detection (LoD) for the two alternative extractions kits was the same as the LoD for the unmodified authorized test (i.e., ≤3xLOD)

The following three conditions were tested.

1. Qiagen kit with new IDT multiplex assay
2. Beckman coulter kit with new IDT multiplex CDC assay
3. ThermoFisher MagMax kit with new IDT multiplex CDC assay

2.3 Equipment and Supplies

2.3.1 Instrumentation and equipment

- Rainin Pipettes
    - Rainin pipettes were used to mix and transfer reagents and samples. Calibrations are performed annually (or as needed) by Rainin,

- Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software
    - This instrument was used to perform PCR testing on clinical specimens. An Operational and Performance Qualification was performed by a qualified ThermoFisher/ABI technician. Testing performance was evaluated with controls included in every test plate run on the instruments during assay verification and actual testing.

2.3.2 Reagents and Materials

2.3.2.1 RNA extraction

Originally, BATL used the CDC approved QIAmp Viral RNA Mini Kit: 250 extractions (52906)

New Extraction kits to be tested were the:

- Beckman Coulter RNAdvance Viral Kit: 768 extractions (C63510)
- ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit: (A48310)

2.3.2.2 Primers and probes

CDC has determined certain commercially available kits of primers and probes may be used with the procedures in CDC-006-00019, Revision: 04, effective 12/01/2020. These kits are listed at https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html. BATL previously performed a bridging study showing the equivalence of the IDT multiplexed CDC assays to the singleplex formats also sold by IDT. For this study BATL used the following multiplex probes and primers;

Manufacturer	**Product Name**	**Catalog No.**
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	nCOV_N1 Forward Primer	10006830
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	nCOV_N1 Reverse Primer	10006831
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	nCOV_N1 Probe (FAM)	10006823

Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	nCOV_N2 Forward Primer	10006833
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	nCOV_N2 Reverse Primer	10006834
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	nCOV_N2 Probe (SUN)	10007050
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	Rnase P Forward Primer	10006836
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	Rnase P Reverse Primer	10006837
Integrated DNA Technologies (IDT) www.idtdna.com (800) 328-2661	Rnase P Probe (ATTO 647)	10007062

2.3.2.3 Positive control

In the context of limited quantities of testing supplies during this public health crisis, based on the best available evidence and in consultation with outside experts, the US Food & Drug Administration (FDA) has determined that certain materials may be used as positive controls:

Control	Manufacturer	Product Name	Catalog Number
N1/N2 Positive Control	Integrated DNA Technologies (IDT), 800-382-2661	2019-nCoV_N_Positive Control	10006625
HS_RPP30 Positive Control	Integrated DNA Technologies (IDT), 800-382-2661	HS_RPP30_Positive Control	10006626

2.3.2.3 RT-PCR Enzyme Mastermix

The following options are available in the CDC instructions for use. BATL used the Thermofisher TaqPath™ 1-Step RT-qPCR Master Mix, CG

Reagent	Quantity	Catalog No.
Quantabio qScript XLT One-Step RT-qPCR ToughMix	100 x 20 µL rxns (1 x 1 mL)	95132-100
	2000 x 20 µL rxns (1 x 20 mL)	95132-02K
	500 x 20 µL rxns (5 x 1 mL)	95132-500
Quantabio UltraPlex 1-Step ToughMix (4X)	100 x 20 µL rxns (500 µL)	95166-100
	500 x 20 µL rxns (5 x 500 µL)	95166-500
	1000 x 20 µL rxns (1 x 5 mL)	95166-01K
Promega GoTaq® Probe 1- Step RT-qPCR System	200 x 20 µL rxns (2 mL)	A6120
	1250 x 20 µL rxns 12.5 mL	A6121
Thermofisher TaqPath™ 1-Step RT-qPCR Master Mix, CG	1000 reactions	A15299
	2000 reactions	A15300

2.4 Data Analysis

2.4.1 Reagent acceptance procedures, criteria and controls

The reagent acceptance procedures, criteria and controls for the original system have been provided by the CDC. They are documented in Standard Analytical Method, SAM 927, “Quality Control of CDC Reagents.” A SAM for multiplex reagent acceptance was developed and approved by the project manager prior to the start of testing.

2.4.2 Test plate acceptance procedures, criteria and controls

A negative template control (NTC) and the appropriate positive control (nCoV_PC, or RPP30_PC) were included in each amplification and detection run.

2.4.3 Range Finding Data

Table 1 shows Ct values obtained for the dilution series prepared using a single positive sample that had a SLD reported Ct value of 24. Each dilution was extracted with the QIAamp Viral RNA mini kit and run using the CDC-EUA assays. The target dilution to use for the equivalency study was identified to be 1:10,000 based on the qualitative detection criteria provided by the CDC instructions for use.

Sample	N1 Avg	N1 Std	N1 Detects		N2Avg	N2 Std	N2 Detects		RP Avg	RP Std	RP Detects
SLD sample (ct24) 1:100	29.51	0.10	3/3		31.08	0.18	3/3		28.07	0.10	3/3
SLD sample (ct24) 1:1,000	32.94	0.51	3/3		34.02	0.37	3/3		28.30	0.08	3/3
SLD sample (ct24) 1:10,000	35.73	1.43	2/3		37.70	0.39	2/3		28.15	0.02	3/3
SLD sample (ct24) 1:100,000	N/A	N/A	1/3		N/A	N/A	1/3		28.15	0.06	3/3
SLD sample (ct24) 1:1,000,000	N/A	N/A	1/3		N/A	N/A	0/3		28.01	0.13	3/3

Table 1 – Range finding to determine the target dilution to use for equivalency testing

2.4.4 Limit of Detection (LOD)

Table 2 shows the comparison of the performance of two alternative viral RNA extraction kits with the original authorized component used in the CDC-EUA assay. As can be seen not only are the qualitative results for all three kits equivalent, but average Ct values together with the standard deviations for every probe used in the CDC assay are very close. The ThermoFisher MagMAX kit used with the automated KingFisher Flex robot was selected for daily testing use and the Beckman RNAdvance kit, which is a manual extraction protocol (96-well) will provide a backup kit in the event there are problems with the high throughput ThermoFisher extraction protocol. This back up kit did have one non-detect seen for the N1 probe. Overall, both kits performed better in terms of limit of detection (1:30,000) than the Qiagen kit.

Qiagen									
Sample	Avg Ct N1	Stdev Ct N1	N1 Detects	Avg Ct N2	Stdev Ct N2	N2 Detects	Avg Ct RP	Stdev Ct RP	RP Detects
SLD sample (ct24) 1:3,000	31.64	0.58	20/20	31.40	0.53	20/20	25.31	0.30	20/20
SLD sample (ct24) 1:10,000	33.21	0.98	20/20	33.33	1.10	20/20	25.33	0.13	20/20
SLD sample (ct24) 1:30,000	34.81	0.82	17/20	35.26	1.39	17/20	25.25	0.29	20/20

Beckman RNAdvance									
Sample	Avg Ct N1	Stdev Ct N1	N1 Detects	Avg Ct N2	Stdev Ct N2	N2 Detects	Avg Ct RP	Stdev Ct RP	RP Detects
SLD sample (ct24) 1:3,000	29.34	0.35	19/20	28.49	0.68	19/20	23.25	0.87	20/20
SLD sample (ct24) 1:10,000	31.31	0.97	20/20	31.05	0.86	20/20	23.21	0.61	20/20
SLD sample (ct24) 1:30,000	33.21	0.61	20/20	33.98	2.36	20/20	23.08	0.16	20/20

ThermoFisher MagMAX									
Sample	Avg Ct N1	Stdev Ct N1	N1 Detects	Avg Ct N2	Stdev Ct N2	N2 Detects	Avg Ct RP	Stdev Ct RP	RP Detects
SLD sample (ct24) 1:3,000	29.69	0.27	20/20	29.15	0.55	20/20	23.80	0.27	20/20
SLD sample (ct24) 1:10,000	32.32	0.67	20/20	32.91	2.03	20/20	24.12	0.36	20/20
SLD sample (ct24) 1:30,000	33.32	0.58	20/20	34.00	1.18	20/20	23.79	0.20	20/20

Table 2 – Comparison of CDC-EUA assays'viral RNA extraction kit to alternative kits

3.0 References

1. **CDC. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19).** https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.
2. **IATA. Shipping Regulations for UN 3373 Biological Substances**, Category B. https://www.iata.org/contentassets/b08040a138dc4442a4f066e6fb99fe2a/dgr-61-en-pi650.pdf.
3. **CDC. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).** https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html
4. **CDC. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel**, CDC-006-00019, Revision: 03, effective 3/30/2020. https://www.fda.gov/media/134922/download
5. **CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel Acceptable Alternative Primer and Probe Sets**. https://www.cdc.gov/coronavirus/2019-ncov/downloads/List-of-Acceptable-Commercial-Primers-Probes.pdf.
6. **FDA. FAQs on Diagnostic Testing for SARS-CoV-2**. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2#whatif
7. **IDT. 2019 Novel Coronavirus (COVID-19) Virus Detection Assay Kit Reagents**. https://www.idtdna.com/pages/landing/coronavirus-research-reagents#references.
8. **RNAdvance Viral XP instructions for Use**. https://www.beckman.com/search#q=c59543&t=coveo-tab-techdocs
9. **QIAGEN. QIAamp Viral RNA Mini Handbook.** https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en

10. **Thermo Fisher. Applied Biosystems. Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument Instructions for Use**. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4406991.PDF,